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Effects of Paweiwan on Streptozotocin-induced Hyperglycemia in Rats

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ABSTRACT

The ancient Chinese remedy of Paweiwan was used by patients with polyuria, polydipsia, and polyphagia. The present study investigated the hypoglycemic effects of Paweiwan using streptozotocin-induced hyperglycemic rats. The effects on serum glucose in a 4-day course, in a 7-week course, on the standard oral glucose tolerance test, and on the liver glycogen content were studied. In the glucose tolerance test, chlorpropamide and insulin were used as the positive controls and 0.5% CMC (Carboxymethylcellulose) was used as the negative control.

We found that Paweiwan decreased the baseline glucose concentration, ameliorated the blood glucose elevation after glucose challenge, and increased liver glycogen content. The results may imply that Paweiwan increases glucose entrance into cells.

Keywords: Paweiwan, streptozotocin

I. Introduction

Diabetes mellitus (DM) is a syndrome characterized by persistent hyperglycemia. Although the pathogenesis of these disorders is diverse, patients ultimately demonstrate an absolute or relative deficiency of insulin (Hindson, 1984). The prevalence of DM clearly has been shown to increase as life patterns change from primitive to civilized modes, and the anticipated rate of increase is 6-7% per year. Today, it is estimated that 5% of all adults in Taiwan are hyperglycemic (Tai *et al.*, 1987). With modern treatments, the morbidity and mortality have been greatly decreased. However, pharmacomedical staff continue to search for new modalities to optimize the treatment of DM. A group of studies have been directed at searching for hypoglycemic agents in Chinese herbs with only partial success: e.g. Rehmanniae Radix (Kitagawa *et al.*, 1971), Corni Fructus (Yamahara *et al.*, 1981), Moutan Cortex (Huang *et al.*, 1980), Hachimigan (Yamahara *et al.*, 1981), and Liu-Wei-Ti-Huang-Tang (Huang, 1985). A similar ancient Chinese remedy, Pawei wan has not been studied. Paweiwan consists of 8 components; Rehmanniae Radix, Corni Fructus, Dioscoreae Batatatis Rhizoma, Moutan Cortex, Alismatis Rhizoma, Poria, Anemarrhenae Rhizoma, and Phellodendre Cortex. The first four components were reported to have hypoglycemic effects. The present study compared the hypoglycemic effects of Paweiwan, the mixture of the four components reported to have hypoglycemic effects (Base I), and the mixture of the other four components reported not to have hypoglycemic effects (Base II), using streptozotocin-induced hyperglycemic rats. The effects of Paweiwan on the standard oral glucose tolerance test and liver glycogen content were also studied. In the oral glucose tolerance test, chlorpropamide and insulin were used as the positive controls and 0.5% CMC (Carboxymethylcellulose) was used as the negative control.

II. Materials and Methods

Preparation of Paweiwan

The Paweiwan was prepared in the following way: a mixture (1 kg) of powders of Rehmanniae Radix, Corni Fructus, Dioscoreae Batatatis Rhizoma, Moutan Cortex, Alismatis Rhizoma, Poria, Anemarrhenae Rhizoma, and Phellodendre Cortex, in a ratio of 8:4:4:3:3:3:2:2, was immersed in 95% alcohol (5000 ml) at room temperature for 3 days. The extract was evaporated and concentrated in a vacuum evaporator at a temperature just below 80°C. The concentrated extract was lyophilized and then pulverized through a No. 80# mesh. The extraction rate was 21%. In Base I, the coarse powders of Remanniae Radix, Corni Fructus, Dioscoreae Batatatis Rhizoma, and Moutan Cortex in a ratio of 8:4:4:3, were mixed and then prepared as described above. The extraction rate was 22%. In Base II, the coarse powders of Alismatis

Rhizoma, Poria, Anemarrhenae Rhizoma, and Phellodendre Cortex in a ratio of 3:3:2 2, were mixed and then prepared as described above. The extraction rate was 18%.

When given to rats or mice, the test drugs (in crude extracts) were dissolved in 0.5% CMC (Carboxymethylcellulose) solution.

Animals

Male Sprague-Dawley rats, weighing between 190 and 230 g, were used in the following studies except in the studies of acute toxicity and effects on the standard oral glucose tolerance test in which male ICR mice weighing between 20 and 25 g were used. The animals were maintained in groups of 6 per cage at a controlled temperature of 22±1°C and humidity of 55 ± 5% and housed in a metal-wire mesh cage. A solid diet (FWU SOW solid diet) and tap water were provided ad libitum.

Control rats or mice were given 0.5% CMC solution.

Acute toxicity

According to the method of Litchfield and Wilcoxon, the LD50s' (95% confidence limits) were assessed 24 hrs after oral or intraperitoneal administration of each preparation to mice.

Measurements of glucose and glycogen contents

Serum glucose was measured by the method of glucose oxidase (Keilin *et al.*, 1948).

The liver glycogen content was measured by a spectrophotometer at a wave length of 620 nm (Seifter *et al.*, 1950).

μg. of glycogen in aliquot was calculated from the following formula:

$$\mu g\cdot \text{ of glycogen in aliquot} = \frac{100 \times U}{1.11 \times S'},$$

where U = the optical density of test solution,

S′ = the optical density of 100-μg· glucose standard,

1.11 = the factor for the conversion of glucose to glycogen.

Effects on serum glucose in a 4-day course

Rats were fasted for 45 hrs and then given streptozotocin (65 mg/kg, i.p.) to induced experimental DM (Yokozawa *et al.*, 1985). Two hrs later, the rats were given Paweiwan, Base I, or Base II (0.5 or 1.0 g/kg, t.i.d., p.o., for 4 days). Blood samples were drawn from tail veins at 10:00 AM after 16 hrs of fasting from the 1st day to the 4th day.

Effects on serum glucose in a 7-week course

Rats were fasted for 45 hrs and then given streptozotocin (90 mg/kg, i.p.) to induce experimental DM (Bonner *et al.*, 1981). Paweiwan, Base I, or Base II (0.5 or 1.0 g/kg, t.i.d.) were given orally from the 3rd week to the 7th week. Blood samples were drawn from tail veins at 10:00 AM after 16 hrs of fasting from the 1st week to the 7th week.

Effects on the standard oral glucose tolerance test

Mice were fasted for 16 hrs and then given streptozotocin (200 mg/kg, i.v.) (Rerup *et al.*, 1969) to induce experimental DM. Two days later, mice were orally given Paweiwan, Base I, or Base II (0.5 or 1.0 g/kg), chlorpropamide (Pfizer, U.S.A., 0.125 g/kg, p.o.), and insulin (Sigma, U.S.A., 0.5, 1.0 units/kg, s.c.). One hr later, the standard oral glucose tolerance test was performed (2 g glucose/kg) (Huang *et al.*, 1983). Blood samples were drawn at 0′, 30′, 60′, 90′, and 120′.

Effects on liver glycogen content

Rats were fasted for 45 hrs and then given streptozotocin (65 mg/kg, i.p.) to induce experimental DM (Yokozawa *et al.*, 1985). Two hrs later, rats were orally given Paweiwan, Base I, or Base II (0.5 or 1.0 g/kg, t.i.d., for 4 days). Rats were fasted from 6:00 PM on the 3rd day. On the 4th day, at 10:00 AM, rats were given the last dose of the test drugs. Three hrs later, rats were decapitated and bled thoroughly. Then 1 g of liver tissue was removed and put in 3 ml 30% KOH solution to measure the liver glycogen content.

Statistical analysis

Results were analyzed statistically using an unpaired Student's *t* test to compare intergroup differences.

III. Results

Acute toxicity

The LD50s' and 95% confidence limits of the 3 preparations in mice are shown in Table 1.

Effects on serum glucose in a 4-day course

As shown in Table 2, streptozotocin induced significant hyperglycemia. Paweiwan showed significant dose-

Table 1. LD50 and 95% Confidence Limits of Paweiwan in Mice

Drugs	Route	LD50(g/kg)	95% confidence limit(g/kg)
P-W	i.p.	4.49	4.17 – 4.83
	p.o.	15.37	10.90 – 21.67
Base I	i.p.	3.42	2.66 – 4.40
	p.o.	12.28	9.82 – 15.35
Base II	i.p.	1.17	1.08 – 1.26
	p.o.	9.20	8.08 – 10.48

Table 2: Effects on Serum Glucose in a 4-day Course

	Dose g/kg p.o.	Serum Glucose (mg/dl) 1st day	2nd day	3rd day	4th day
Normal +0.5% CMC		101±5	100±7	105±6	102±8
Normal +P-W	0.5	102±6	105±5	113±5	107±6
STZ + 0.5% CMC		248±19	454±9	480±15	482±16
STZ + P-W	0.5	216±12	438±10	413±10↓↓	398±12↓↓
	1.0	264±20	395±18↓	390±8↓↓↓	380±11↓↓↓
STZ + Base I	0.5	251±18	443±9	430±18↓	414±22↓
	1.0	229±10	413±15↓	415±17↓	397±11↓↓
STZ + Base II	0.5	269±16	450±4	462±18	440±40
	1.0	219±10	460±29	mortality (2/6)	mortality (3/6)

1. STZ: streptozotocin (65 mg/kg, i.p.), P-W: Paweiwan
2. STZ + P-W, STZ+Base I, STZ + Base II were compared with STZ + 0.5% CMC
3. mean ± S.E. (n = 6)
4. ↓: $P < 0.05$, ↓↓: $P < 0.01$, ↓↓↓: $P < 0.001$

dependent hypoglycemic effect ($P < 0.05$ to 0.001) in experimental DM rats. However, it had no significant hypoglycemic effect in normal rats. Base I had weaker hypoglycemic effect ($P < 0.05$ to 0.01) than that of Paweiwan. Base II had no hypoglycemic effect and caused death in some rats on the 3rd and the 4th day.

Effects on serum glucose in a 7-week course

As shown in Figure 1, streptozotocin induced significant hyperglycemia during the 7 weeks of experiment. The 0.5% CMC solution had no apparent effect on fasting serum glucose. Paweiwan showed a dose-dependent hypoglycemic effect ($P < 0.05$ to 0.001). Base I showed weaker hypoglycemic effect ($P < 0.05$) than that of Paweiwan. Base II had no apparent effect on fasting serum glucose and caused death in some rats after the 6th week.

Effects on the standard oral glucose tolerance test

As shown in Table 3, streptozotocin induced significant elevation of plasma glucose after oral glucose challenge. Paweiwan could significantly ameliorate the elevation of plasma glucose ($P < 0.05$ to 0.001) from 30′ to 120′. Base I could not ameliorate the elevation of plasma glucose. Base II might even augment the elevation of plasma glucose ($P < 0.05$ to 0.01) from 90′ to 120′.

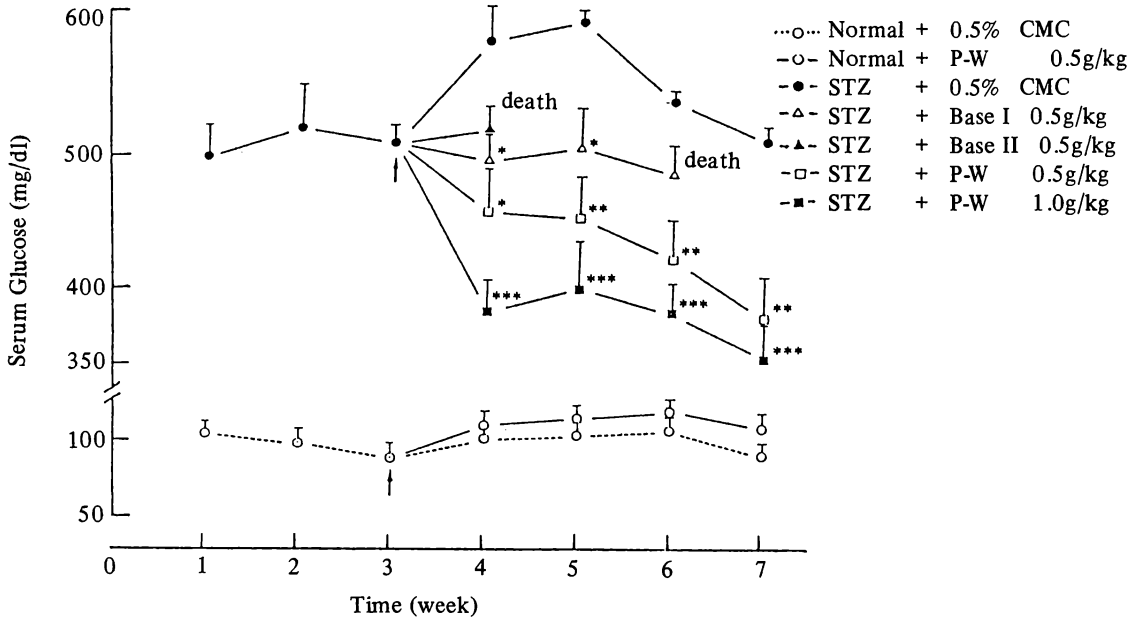

Fig. 1. Effects on serum glucose in a 7-week course
1. STZ: steptozotocin (90 mg/kg, i.p.), P-W: Paweiwan
2. STZ+Base I, STZ+P-W(0.5 g/kg), STZ+P-W(1.0 g/kg) were compared with STZ+0.5% CMC
3. mean ± S.E. (n = 6)
4. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$

Table 3. Effects on the Standard Oral Glucose Tolerance Test

	Dose g/kg p.o.	Serum Glucose in OGTT (mg/dl) 0′	30′	60′	90′	120′
Normal +0.5% CMC		84±4	301±15	209±18	158±8	149±9
STZ + 0.5% CMC		466±15	633±14	618±18	590±16	577±16
STZ + P-W	0.5	484±15	575±15↓	529±18 ↓↓	532±18 ↓	515±18↓
	1.0	459±19	509±15↓↓↓	524±18↓↓	501±19↓↓	494±19↓↓
STZ+Base I	0.5	476±16	663±14	620±17	609±17	577±16
	1.0	471±18	630±13	613±15	596±26	576±19
STZ+Base II	0.5	503±13	655±15	661±18	657±22↑	656±17↑↑
	1.0	511±15	610±29	595±14	573±16	551±26
Chlorpropamide	0.125	479±18	623±20	614±18	580±15	562±16
Insulin	0.5units/kg	425±14	502±13↓↓↓	497±13↓↓↓	490±18↓↓↓	475±12↓↓↓
	1.0units/kg	314±10↓↓↓	452±14↓↓↓	423±12↓↓↓	324±12↓↓↓	258±8 ↓↓↓

1. STZ: streptozotocin (200 mg/kg, i.p.), P-W: Paweiwan
2. STZ+P-W, STZ+Base I, STZ+Base II were compared with STZ+0.5% CMC
3. mean ± S.E. (n = 10)
4. ↓: $P<0.05$, ↓↓: $P<0.01$, ↓↓↓: $P<0.001$, ↑: $P<0.05$, ↑↑: $P<0.01$

Table 4. Effects on Liver Glycogen Contents

	Dose g/kg, p.o.	Glycogen Content (μg/gm liver in aliquot) by Direct Anthrone Procedure
Normal +0.5% CMC		23.5±1.2
STZ + 0.5% CMC		19.1±1.0
STZ+P-W	0.5	26.6±1.6↑↑
	1.0	27.6±1.7↑↑
STZ+Base I	0.5	19.5±1.0
	1.0	22.2±1.1
STZ+Base II	0.5	16.4±0.7 ↓

1. STZ: streptozotocin (65 mg/kg, i.p.), P-W: Paweiwan
2. STZ+P-W, STZ+Base I, STZ+Base II were compared with STZ+0.5 CMC
3. mean ± S.E. (n = 6)
4. ↓: $P<0.05$, ↑↑: $P<0.01$

Chlorpropamide had no hypoglycemic effect. But insulin (0.5, 1.0 units/kg) had stronger hypoglycemic effect ($P < 0.001$) than that of Paweiwan.

Effects on liver glycogen content

As shown in Table 4, streptozotocin could significantly decrease the liver glycogen content. Paweiwan could significantly ameliorate the decrease in liver glycogen content ($P < 0.01$). Base I had no significant effect on liver glycogen content. Base II could decrease the glycogen content ($P < 0.05$).

IV. Discussion

In the present studies, we used 95% alcohol as the solvent to extract the effective constituents because it was reported that Kue-Fu-Pa-Wei-Wan, a remedy with similar composition to that of Paweiwan, had no antihyperglycemic effect if water was used as the solvent (Yamahara *et al.*,1981).

Both alloxan and streptozotocin have been used to induce experimental diabetes mellitus. In the present study, we used streptozotocin because it was reported that the effects of alloxan were more influenced by the sex, age, and nutritional status of rats (Clin *et al.*, 1984). Furthermore, male rats, instead of female rats, were used in the present study because menstrual cycles in female rats may cause greater blood glucose fluctuation (Clin *et al.*, 1984).

In previous work in searching for antihyperglycemic agents among Chinese herbs, only a blood glucose sample was used as the index, after 4 days of a single oral test dose (Yamahara *et al.*, 1981) or after 6 days of a single oral test dose (Yokozawa *et al.*, 1985). However, we measured blood glucose levels every day for 4 days or every week for 4 weeks. The trend in blood glucose changes may be a more accurate observation.

Furthermore, the standard oral glucose tolerance test (Huang *et al.*, 1983) was also used to observe the blood glucose changes. Under the very standardized conditions, we observed the blood glucose changes after the administration of Paweiwan, Base I, or Base II. Liver glycogen contents were used to observe whether the lowering of blood glucose was due to glucose entering the cells.

Although, in the oral glucose tolerance test, Paweiwan showed hypoglycemic effects weaker than that of insulin but stronger than that of chlorpropamide, the animal model could not be directly applied to human beings. The therapeutic index of Paweiwan was narrow, so clinical application of the remedy should be very careful.

From the studies on blood glucose and liver glycogen contents, the results were all comparable and im-

plied that Paweiwan increased glucose entering cells. And Base II had synergistic hyperglycemic effects on Base I. The mechanism of the synergistic effect may be due to the chemical reactions in the preparation of Paweiwan creating a new compound which has a stronger hypoglycemic effect.

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References

Bonner-Weir, S., D.F. Trent, R.N. Honey and G.C. Weir. (1981) Responses of neonatal rats islets to streptozotocin limited β-cell regeneration and hyperglycemia. *Diabetes*, 30, 64-69.

Clin, H., L.H. Mei, I.L. Seng and C.L. Chun. (1984) The hypoglycemic effect of the crude drug prescription resources from Taiwan on the experimental diabetes mellitus (I). *J. Taiwan Pharm. Assoc.*, **36(3)**, 160.

Tai T.Y., C.J. Chen, C.L. Yang and L.S. Ko. (1987) Epidemiology of diabetes mellitus in Taiwan. *J. Med. Ass. Thailand*, **70**, 42-48.

Hindson, D.A. (1984) Diagnosis and treatment of diabetes mellitus. In: Drug treatment in the elderly, ADIS Health Science Press, 221-234, (Robert, E.V., Ed), Bristol.

Huang, R.L., J.H. Lin and K.L. Liu. (1980) Studies on the antihyperglycemic action of Chinese drugs and herbs (III). The Annual Reports of the National Research Institute of Chinese Medicine, p. 13.

Huang, R.L., J.H. Lin and K.J. Liu. (1983) Studies on the antihyperglycemic action of Chinese drugs and herbs (VI). The Annual Reports of the National Research Institute of Chinese Medicine, p. 17-29.

Huang, R.L. (1985) Studies on the antihyperglycemic action of Chinese drugs and herbs (VIII). The Annual Reports of the National Research Institute of Chinese Medicine, p. 17.

Keilin D. and E.F. Hartree. (1948) Properties of glucose oxidase (Notatin). *Biochem. J.*, **42**, 221.

Kitagawa, I., T. Nishimura, A. Furubashi and I. Yosioka. (1971) On the constituents of Rhizome of Rehmannia glutinosa Lirosch. forma hueichinhensis Hsiao. *Yakugaku Zasshi*, 91 (5), 593-596.

Rerup C. and F. Tarding. (1969) Streptozotocin- and Alloxan-diabetes in mice. *European J. Pharmacol.*, 7, 89-96.

Seifter, S., S. Dayton, B. Novic and E. Muntwyler. (1950) The estimation of glycogen with the anthrone reagent. *Arch. Biochem.*, **25**, 191.

Yamahara, J., H. Mibu, T. Sawada, H. Fujiumura, S. Takino, M. Yoshikawa and I. Kitagawa. (1981) Biologically active principles of crude drug. Antidiabetic principles of Corni Fructus in experimental diabetes induced by streptozotocin. *Yakugaku Zasshi*, **101(1)**, 86.

Yokozawa, T., K. Kobagashi, H. Oura and Y. Kawashima. (1985) Hyperlipemia-Improving effects of Ginsenoside-Rb_2 in streptozotocin-diabetic rats. *Chem. Pharm. Bull.*, **33(9)**, 3893-3898.

Yokozawa, T., T. Kobayashi, H. Oura and Y. Kawashima. (1985) Studies on the mechanism of the hypoglycemic activity of Ginsenoside-Rb_2 in streptozotocin-diabetic rats. *Chem. Pharm. Bull.*, **33(2)**, 869.

知柏八味丸對於Streptozotocin誘發高血糖大白鼠之影響

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摘　要

知柏八味丸由熟地黃、山茱萸、山藥、牡丹皮、澤瀉、茯苓、知母、黃柏等藥材所組成，在中醫臨床上用來治療多尿、多渴、多飢的病人。而本研究係探討知柏八味丸，Base I (熟地黃、山茱萸、山藥、牡丹皮)，Base II (澤瀉、茯苓、知母、黃柏) 對於 Streptozotocin 誘發高血糖大白鼠之影響，探討其於四天內血糖濃度、於七星期內血糖濃度、標準口服葡萄糖耐量試驗與肝醣之測定。於標準口服葡萄糖耐量試驗中，使用 chlorpropamide 及 insulin 爲對照品，比較知柏八味丸之效果。

由實驗結果顯示知柏八味丸具有降血糖作用、增加葡萄糖利用能力與增加肝醣合成量。因此我們推論知柏八味丸具有增加葡萄糖進入細胞的作用。又 Base I 配合 Base II 後爲八味丸其降血糖作用增加及毒性減低，可能由於其成分間產生相互作用，此有待進一步探討其成分變化。